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Research Article

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Androstenedione and Its Conversion to Plasma Testosterone in Congenital Adrenal Hyperplasia *

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Summary. The plasma concentration, production rate, and conversion ratio of androstenedione and testosterone were studied in seven children with congenital adrenal hyperplasia (CAH) of the 21-hydroxylase type. Plasma androstenedione and testosterone measured by double isotope derivative assay and estimated blood production rates were manyfold increased in the untreated state, markedly suppressed with glucocorticoid, and increased after the administration of ACTH.

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Introduction

The major clinical manifestation in patients with congenital adrenal hyperplasia (CAH) of the 21-hydroxylase type is progressive virilization. These abnormalities can be reproduced by the administration of androgenic steroids. In this disorder, an enzyme deficiency in cortisol synthesis and secretion leads to hypersecretion of adreno-

corticotropin (ACTH), which partially overcomes the block, but at the expense of increased secretion of steroid precursors among which are androgenic steroids (1-3).

The nature of the androgenic substance responsible for the clinical manifestations of adrenal hyperplasia has not been clarified. Since testosterone is the most potent natural androgenic hormone and the adrenal is capable of synthesizing testosterone (4), increased secretion of this steroid has been suggested as the responsible mechanism. This hypothesis is supported by the demonstration of increased urinary excretion (5-11), plasma concentration (10, 12-14), and production rate (10-12) of testosterone in patients with congenital adrenal hyperplasia. However, recent work by Korenman and Lipsett (15) and Horton and Tait (16) indicates that the weak androgen, androstenedione (androst-4-ene-3,17-dione), can be converted by peripheral tissue *in vivo* to testosterone. Horton and Tait have fur-

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ther demonstrated that almost two-thirds of blood testosterone in the normal female is derived from blood androstenedione. Since an analogous situation might be present in certain clinical states, this study was designed to further explore the *in vivo* mechanism of virilization in congenital adrenal hyperplasia.

Methods

Three prepubertal females and four prepubertal males with congenital adrenal hyperplasia were studied. Two children were previously untreated (R.D., F.D.), and five were studied 4 to 6 weeks after suppressive therapy had been discontinued. All patients were significantly virilized in the untreated state with advanced bone age. The diagnosis of congenital adrenal hyperplasia was based on progressive virilization, increased excretion of 17-ketosteroids and pregnanetriol, normal or low 17-hydroxysteroid excretion (Porter-Silber chromogens), and urinary ketosteroid suppression with physiological doses of cortisol or cortisol analogue. Therefore, all patients are considered to have a 21-hydroxylase block.

Urinary 17-ketosteroids were determined by a modification of the method of Dreker and associates (17), and urinary pregnanetriol was measured by the technique of Cox (18). Normal values for prepubertal children are less than 5 mg per day and less than 2 mg per day, respectively.

Plasma testosterone and androstenedione were measured by double isotope derivative techniques using thiosemicarbazide-³⁵S and steroid-1,2-³H indicator of high specific activity. Testosterone was measured by the method of Riondel and co-workers (19) and androstenedione by the method of Horton (20). The non-specific blank, accuracy, and sensitivity are as originally described. Greater experience with these techniques has resulted in an equivalent or increased recovery with half the original amount of reagent used (0.5 mg, SA 140 to 160 mc per mmole). This improvement, however, has

no bearing upon sensitivity and accuracy, which are primarily dependent upon the variation of the nonspecific blank (21).

The metabolic clearance rate of androstenedione and testosterone and their conversion ratios in blood were determined by constant infusion of tritiated steroid and isolation of infused steroid and its conversion product from plasma as previously described (16). The infusions were all performed between 8 and 11 a.m. The metabolic clearance rate was calculated as the rate of infusion of ³H per day divided by the plasma radioactivity as the infused steroid per liter at equilibrium corrected for recovery. The blood production rate, which is the total amount of hormone entering the general circulation, was calculated as the product of the plasma concentration and the metabolic clearance rate (16). A diurnal variation in plasma testosterone, although of lesser magnitude than cortisol, has been noted in normal adult male subjects (22). Therefore the mean calculated testosterone blood production rate is an estimation of the actual daily blood production rate.

The conversion ratio in blood was calculated as previously described (16) as the ratio of counts per minute ³H per liter corrected for recovery, of product to precursor. Previous work has demonstrated that equilibrium is obtained in blood after infusion of ³H-labeled androstenedione and testosterone for 130 minutes and that the chromatographic systems result in radiochemical isolation of both these steroids from plasma (16).

The recoveries, levels of counting, and counting errors of this approach are described in the original paper (16) and extensively discussed in a recent report by Horton and Tait (23).

Results

Urinary steroids. The urinary steroid excretions of the children with CAH, in the untreated state and after cortisol suppression, are shown in Table I. The mean excretion of 17-ketosteroids and pregnanetriol in the untreated state was 17 mg per day. No sex differences in these values were noted in these children with CAH.

Treatment with cortisol resulted in a fall in the mean 17-ketosteroid value to 3.7 mg per day. In all subjects the ketosteroid values reached normal or subnormal levels on low doses of cortisol (20 to 30 mg per day).

Plasma concentration of androstenedione and testosterone in congenital adrenal hyperplasia. The mean concentration of androstenedione (corrected for the mean blank of 17 m μ g per 100 ml) was 380 m μ g per 100 ml for males and 647 m μ g per 100 ml for females with CAH. Mean plasma testosterone (corrected for the mean blank of 19 m μ g per 100 ml) was 144 m μ g per 100 ml for

TABLE I
Urinary steroid excretion in congenital adrenal hyperplasia (CAH)

| Patient | Age | Sex | Untreated | | Cortisol suppression |
|----------|-------|-----|---------------------|----------------|----------------------|
| | | | Urinary ketosteroid | Preg-nanetriol | Ketosteroid |
| | years | | mg/day | | mg/day |
| 1. F.D. | 4 | M | 16 | 14 | 5.1 |
| 2. M.P.* | 6 | M | 10 | 19 | 3.4 |
| 3. R.S. | 10 | M | 14 | 15 | 4.2 |
| 4. R.M. | 8 | M | 21 | 18 | 4.8 |
| 5. R.D. | 8 | F | 25 | 19 | 3.3 |
| 6. S.S. | 9 | F | 14 | 13 | 3.4 |
| 7. D.C. | 7 | F | 21 | 20 | 4.1 |

* Salt loser.

TABLE II
Plasma concentration of A and T, conversion ratios in blood, and contribution of A to T in CAH

| Patient | Sex | Plasma concentration in untreated patients | | CR _{BB} ^A T* | T from A | % Total | T from "T"† | Plasma concentration after cortisol suppression | |
|---------|-----|--|--------------|----------------------------------|-------------------|---------|-------------|---|--------------|
| | | Androstenedione | Testosterone | | | | | Androstenedione | Testosterone |
| | | <i>mμg/100 ml</i> | | | <i>mμg/100 ml</i> | | | <i>mμg/100 ml</i> | |
| 1. F.D. | M | 220 | 120 | 0.17 | 36 | 30 | 84 | 0 | 20 |
| 2. M.P. | M | 320 | 130 | 0.14 | 45 | 35 | 85 | 28 | 58 |
| 3. R.S. | M | 320 | 134 | 0.13 | 42 | 31 | 92 | 19 | 70 |
| 4. R.M. | M | 660 | 190 | 0.14 | 93 | 49 | 97 | 56 | 19 |
| 5. R.D. | F | 670 | 110 | 0.14 | 94 | 86 | 16 | 23 | 5 |
| 6. S.S. | F | 720 | 180 | 0.17 | 122 | 68 | 48 | 90 | 0 |
| 7. D.C. | F | 550 | 120 | 0.16 | 88 | 73 | 32 | 18 | 0 |
| Mean | M | 380 | 144 | 0.15 | 54 | 36 | 89 | 26 | 42 |
| Mean | F | 647 | 137 | | 101 | 76 | 32 | 43 | 2 |

* Conversion ratio, androstenedione (A) to testosterone (T), calculated as the ratio of mean androstenedione-³H and testosterone-³H (Table III).

† Amount of T in plasma unaccounted for by peripheral conversion of A.

males and 137 mμg per 100 ml for females with CAH (Figure 1 and Table II).

Plasma concentrations in male children with congenital adrenal hyperplasia receiving cortisol were 26 mμg per 100 ml androstenedione and 42 mμg per 100 ml testosterone, and in females were 43 mμg per 100 ml androstenedione and 2 mμg per 100 ml (essentially undetectable) testosterone (Table II).

Metabolic clearance rate (MCR). The metabolic clearance rate of androstenedione-³H (MCR^A) in children with CAH is presented in Table III. The mean MCR^A was 1,030 L per day in three female children and 844 L per day in four male children. Since the children varied considerably in age and body size and these values could not be compared with adult values, they were corrected for body surface. The mean MCR^A was 1,027 L per day per m² for females and 934 L per day per m² for male children. There was no significant sex difference in the two groups, and the combined MCR^A was 970 ± 45 (SE) L per day per m² (seven subjects). Adult values when corrected for body surface are 1,100 ± 50 (SE) L per day per m² (twelve subjects) based upon the normal adults studied by Horton and Tait (16) when corrected for body surface area.

Evidence for attaining equilibrium is based upon analysis of radioactivity present at 105 and 120 minutes after the start of the infusion. Taking the value for 105 minutes (135 minutes after load-

ing dose) as 100%, the mean value at 120 minutes was 102 ± 3 (SE) % for androstenedione and 98 ± 3.9 (SE) % for testosterone after androstenedione-³H infusion.

The metabolic clearance rate of testosterone (MCR^T) in two children [one male (M.P.), one female (S.S.)] with CAH was 322 and 324 L per day per m².

Production rate in blood. The mean production rate in blood of androstenedione in untreated

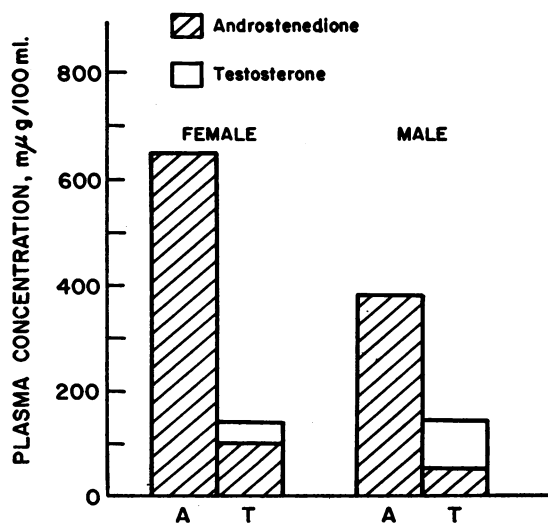


FIG. 1. ANDROSTENEDIONE AND TESTOSTERONE CONCENTRATION IN PLASMA IN CONGENITAL ADRENAL HYPERPLASIA. The hatched area in the testosterone column represents the contribution of androstenedione by peripheral conversion.

TABLE III
The metabolic clearance rate and interconversion in blood of androstenedione to testosterone in CAH

| Patient | Sex | Age years | A→H infused cpm/day | Androstenedione- ³ H | | | MCR ^A * | Surface area m ² | MCR ^A | Testosterone- ³ H | | |
|---------|-----|--------------|---------------------------|---------------------------------|---------|--------|--------------------|-----------------------------------|----------------------|------------------------------|---------|-------|
| | | | | 130 min | 145 min | Mean | | | | 130 min | 145 min | Mean |
| | | | | cpm/L | | | L/day | | L/day/m ² | cpm/L | | |
| 1. F.D. | M | 4 | 32.0 ×10 ⁶ | Pool | | 43,600 | 735 | 0.72 | 1,020 | Pool | | 7,420 |
| 2. M.P. | M | 6 | 17.5 ×10 ⁶ | 28,500 | 29,000 | 28,750 | 610 | 0.70 | 870 | 4,330 | 3,750 | 4,040 |
| 3. R.S. | M | 10 | 25.8 ×10 ⁶ | 28,000 | 31,200 | 29,600 | 870 | 1.01 | 860 | 3,680 | 3,770 | 3,725 |
| 4. R.M. | M | 8 | 19.3 ×10 ⁶ | 17,000 | 16,200 | 16,600 | 1,160 | 1.18 | 985 | 2,400 | 2,360 | 2,380 |
| 5. R.D. | F | 8 | 26.7 ×10 ⁶ | 23,000 | 21,600 | 22,300 | 1,200 | 1.06 | 1,130 | 3,200 | 2,900 | 3,050 |
| 6. S.S. | F | 9 | 26.64 ×10 ⁶ | 22,000 | 23,800 | 22,900 | 1,150 | 1.10 | 1,045 | 4,600 | 5,000 | 4,800 |
| 7. D.C. | F | 7 | 30.1 ×10 ⁶ | Pool | | 40,700 | 740 | 0.82 | 905 | Pool | | 6,500 |
| | | | | | | | | | Mean | 934 male | | |
| | | | | | | | | | | 1,027 female | | |
| | | | | | | | | | | Mean combined | | |
| | | | | | | | | | | 970 ±45 (SE) | | |

* MCR^A = metabolic clearance rate of androstenedione-³H.

adrenal hyperplasia, calculated as the product of concentration and metabolic clearance rate, was 6.3 mg per day per m² in female children and 3.7 mg per day per m² in male children. With a metabolic clearance rate of 320 L per day per m², the mean blood production rate of testosterone in untreated adrenal hyperplasia was 0.44 mg per day per m² in female children and 0.46 mg per day per m² in male children.

After cortisol suppression, the calculated blood production of androstenedione was less than 0.5 mg per day per m² in all groups. Production rate in blood of testosterone after treatment was unmeasurable in female children and 0.21 mg per day per m² in prepubertal male children with CAH.

Conversion ratios in blood in CAH. The conversion ratio of androstenedione to testosterone in blood (CR_{BB}^{AT}) in seven children with CAH was 15 ± 0.6 (SE) % (Table II). There was no sex difference in the children studied. The conversion rate in normal adults has been measured at 14 ± 1 (SE) % (16).

The back conversion of testosterone to androstenedione was 3.4% and 1.0% in two prepubertal CAH children. The CR_{BB}^{TA} in adults is 2.8 ± 0.2 (SE) % (16).

The contribution of plasma androstenedione to plasma testosterone in untreated congenital adrenal hyperplasia. The product of plasma androstenedione concentration and the conversion ratio (CR_{BB}^{AT}) yields the concentration of plasma testosterone derived from plasma androstenedione, and this with the total concentration of plasma

testosterone in the same sample allows calculation of the per cent of plasma testosterone derived from plasma androstenedione from peripheral conversion. This calculation is simplified by the finding that the back conversion is insignificant in terms of actual contribution to androstenedione concentrations in blood. In the prepubertal females with CAH, 101 mμg per 100 ml testosterone, and in the male, 54 mμg per 100 ml testosterone was derived from the peripheral conversion of androstenedione. This is 76% and 36% of total plasma testosterone in female and male children with untreated CAH, respectively (Table II). The remaining amount of testosterone in plasma unaccounted for by peripheral conversion of androstenedione (T from "T," Table II) was 32 mμg per 100 ml in the female and 89 mμg per 100 ml in the males with CAH.

The effect of ACTH on plasma androgens in CAH. To determine the effect of ACTH on the virilizing levels of plasma androgen, we gave three prepubertal subjects (two males and one female), suppressed with cortisol for 4 weeks, iv infusions of ACTH, 25 U in 360 ml 5% dextrose in water for 6 hours.

There was a rise in both plasma androstenedione and testosterone in all three subjects. In two, the final values were similar to those noted in the untreated state (Table IV). In the third patient, R.S., a 10-year-old male treated for 7 years, there initially was only a minimal rise in plasma androgens after ACTH. He was then given ACTH gel, 40 U every 12 hours for 4 days followed by another iv ACTH infusion. The con-

TABLE IV
Plasma androgens in CAH after ACTH

| Pa- tient | Sex | Cortisol suppression | | ACTH (25 U iv for 6 hours) | | |
|--------------|-----|----------------------|--------------------------|-------------------------------|--------------|--|
| | | Andros- tenedione | Testosterone | Andros- tenedione | Testosterone | |
| | | | <i>mμg/100 ml plasma</i> | | | |
| S.S. | F | 10 | 45 | 480 | 137 | |
| R.S. | M | 83 | 29 | 202 | 70 | |
| R.M. | M | 60 | 20 | 741 | 143 | |

tinued administration of ACTH resulted in a moderate elevation in plasma androgen values.

Discussion

Testosterone secretion in men and women contributes only a small fraction of the total urinary 17-ketosteroids (24, 25). Any meaningful analysis requires measurement of the androgen concentration and production rates in blood since the $C_{19}O_2$ steroids are interconvertible in the body. Recent work by Horton, Shinsako, and Forsham (26) and Tait and Horton (27) has indicated that in the normal adult female there is a gross difference between the production rate of testosterone in blood (0.4 mg per day) and the production rate calculated from the urinary metabolite (1.5 mg per day). Mahesh and Greenblatt (28), Camacho and Migeon (25), and Korenman and Lipsett (15) demonstrated that androstenedione and dehydroisoandrosterone can be converted *in vivo* to testosterone glucuronoside. Horton and Tait then measured plasma androstenedione and testosterone concentrations, metabolic clearance, and the interconversion rates and concluded that almost two-thirds of plasma testosterone in the female is derived from peripheral conversion of secreted androstenedione (16). Dehydroisoandrosterone appears to be a minor source of plasma testosterone (23). This new information suggested that an analogous situation might be present in a disorder such as congenital adrenal hyperplasia. In this study of children with congenital adrenal hyperplasia and virilization, plasma testosterone was considerably elevated in all patients regardless of sex or age.

The normal values for testosterone are 800 ± 70 (SE) $m\mu g$ per 100 ml in adult males and 34 ± 8 (SE) $m\mu g$ per 100 ml for females by the thio-

semicarbazide- ^{35}S method (13, 19). Values in normal prepubertal children are 42 ± 9 (SE) $m\mu g$ per 100 ml in males and 19 ± 9 (SE) $m\mu g$ per 100 ml in normal prepubertal females (29). Children with CAH have testosterone concentrations four times higher than adult females and seven times greater than normal prepubertal children of comparable age and sex.

Normal values for androstenedione are 60 ± 4 (SE) $m\mu g$ per 100 ml for adult males and 140 ± 8 (SE) $m\mu g$ per 100 ml in adult females (20). Prepubertal values are 86 ± 12 $m\mu g$ per 100 ml in males and 30 ± 4 $m\mu g$ per 100 ml in females (29). Female children with CAH have a four-fold elevation in blood androstenedione compared to adult female values, and a fifteenfold elevation compared to normal prepubertal values. Male children with CAH have values for androstenedione averaging four times greater than normal prepubertal values. There is a marked increase in both plasma androstenedione and testosterone concentrations in children with untreated CAH.

The role of ACTH in the hypersecretion of these androgenic steroids is suggested by the suppression of circulating androstenedione and testosterone levels after cortisol administration and stimulation of circulating androgens by ACTH in patients suppressed with cortisol. Testosterone values after suppression in prepubertal females with CAH are essentially undetectable. Plasma testosterone concentrations in suppressed male children are similar to normal prepubertal male values. ACTH increased values for androstenedione and testosterone in three cortisol-suppressed CAH children. In two subjects, the concentrations present in the untreated state were reproduced; in the third only a moderate rise was obtained even after 4 days of parenteral ACTH (25 U twice a day im) and an iv infusion for 6 hours (25 U). The latter patient had been on suppressive treatment for over 6 years, and secondary adrenal atrophy perhaps is the explanation for this blunted response.

The metabolic clearance rates of androstenedione do not appear significantly different from the normal adult values when expressed in liters per day per square meter. The MCR^A values of 970 ± 45 (SE) L per day per m^2 do not demonstrate a sex difference as has been reported in normal adults (16).

The blood production rates of androstenedione and testosterone in normal adults have been recently calculated from the plasma concentration and metabolic clearance rates. Blood production of androstenedione is 3.3 mg for the female and 1.4 mg per day for males; the blood production of testosterone is 0.4 mg and 6 mg per day, respectively (16). The blood production rates of androstenedione in CAH expressed as milligrams per day per square meter are almost three times the normal adult values and even more increased as compared with estimated production rates of normal prepubertal children. Blood production rates of testosterone can only be approximated in CAH children because of diurnal variation, and a more accurate determination should be based upon larger numbers of clearance determinations. Nevertheless, the estimated blood testosterone production rates in CAH (0.4 mg per day per m²) are equal to or greater than the maximal calculated testosterone blood production rates in adult females (26, 27) and at least threefold increased when compared with estimated values in prepubertal children.

The conversion ratio of androstenedione to testosterone (CR_{BB}^{AT}) calculated as the corrected ratio of product to precursor steroid at equilibrium in counts per minute per liter has been reported as 0.14 ± 0.01 (SE) in normal adults (16). The CR_{BB}^{AT} in seven children with CAH is 0.15 ± 0.01 (SE) (Table II). The conversion ratio of androstenedione to testosterone in CAH is not significantly different from normal adult values.

The transfer constant or rho value ($[\rho]_{BB}^{AT}$) is defined as the fraction of precursor entering blood that enters the blood as the product. This value can be calculated as the product of the conversion ratio and the ratio between metabolic clearance of product and precursor: counts per minute per day ³H product entering blood/counts per minute per day ³H precursor entering blood = (counts per minute per liter product \times MCR product)/(counts per minute per liter precursor \times MCR precursor) = $CR_{BB}^{AT} \times (MCR^P/MCR^A)$. When $[\rho]^{AT}$ values are determined in this way in CAH using MCR in liters per day per square meter, the mean transfer constant for androstenedione to testosterone in blood is 5%, which is not significantly different from this determination in

normal adult subjects, $[\rho]_{BB}^{AT} = 5.9 \pm 0.5$ (SE) % (16).

Consideration of the markedly increased concentration of androstenedione and the conversion ratios indicates that about two-thirds of the testosterone in the female children with CAH and about one-third in the males are derived from peripheral conversion of blood androstenedione. The difference in the two sexes may be more apparent than real, since the remaining concentration of testosterone in plasma after subtraction of the androstenedione contribution (T from "T") is of the same order of magnitude as normal prepubertal values in the respective sexes (29). The data suggest that almost all of the increased testosterone in plasma in children with CAH and virilization is the result of increased androstenedione secretion.

Camacho and Migeon have recently measured the excretion of testosterone glucuronoside and calculated the production rate from the integrated specific activity of the urinary metabolite (Tg) in patients with CAH. The excretion and calculated production rates are increased in CAH when compared with normal controls. The production rate of testosterone from the urinary metabolite in the single prepubertal female studied was 2.78 mg per day (11). If this value and our studies are representative of the disorder before puberty, it can be calculated that about 2.4 mg of testosterone glucuronoside (Tg) should be derived from blood androstenedione using the over-all conversion of androstenedione to urinary testosterone of 40% (23, 26) (6.3 mg androstenedione per day \times 0.4 = 2.4 mg Tg per day). This would indicate that almost all of the testosterone production rate calculated from the urinary metabolite of testosterone is derived from blood androstenedione in CAH. This is further evidence that the direct secretion of blood testosterone in the female child with CAH is minimal.

The major role in CAH of androstenedione secreted by the adrenal cortex under the influence of ACTH appears to be established. Virilization arises from the marked increase in blood testosterone production resulting in large part from the peripheral conversion of abnormal amounts of the secreted precursor androstenedione.

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